Faculdade de Medicina Dentária da Universidade do Porto

Salivary biomarkers of dental caries

A Systematic Review

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Porto 2013

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Artigo de Revisão Bibliográfica apresentado no
âmbito da UC "Monografia de Investigação"
do Mestrado Integrado de Medicina Dentária

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Porto 2013

Index

1. Abstract

Aim: Saliva can be used to study the physiological state of the body, having the potential to be used in the early detection and diagnosis of diseases. This is due to the abundant protein content in saliva, which can behave as biomarkers for diseases. The present study aimed to compose a systematic review about the potential salivary biomarkers of dental caries.

Methods: Scientific manuscripts were searched in PUBMED, ScienceDirect, MEDLINE, BIOMED databases, which related salivary biomarkers with dental caries, using several keywords and MeSH terms. The present review includes articles published in majority between 2000 and 2013.

Results: Many proteins are referred in scientific literature as potential biomarker of dental caries. This review includes the most important information regarding some of them: low levels of soluble immunoglobulin A are present in high caries susceptibility groups; the inverse relationship between Mucins 1 and 2 levels and the prevalence of dental caries; high levels of statherin and cystatin S in caries-free individuals; the association between ACG haplotype of DEFB 1 and a high decayed, missing, or filled teeth or decayed, missing, or filled teeth surfaces; the inverse relationship between the presence of sCD14 and caries lesions; the increase in the glucosyltransferase B level with the increase of caries experience.

Conclusion: There are various proteins candidate for a biomarker role on dental caries, namely, soluble immunoglobulin A, mucins 1 and 2, cystatin S, statherins, defensins, CD14 and glucosyltransferase B. However, further studies are needed to define a benchmark to infer whether the individual has an increased risk of caries. This could open doors for an actuation in an early stage of the disease.

Keywords: salivary proteins AND caries, oral biomarkers AND caries, salivary diagnosis AND caries, salivary proteome, salivary biomarkers.

2. Introduction

Dental caries is one of the most common preventable childhood diseases, which is often not self-limiting and without proper care, caries can progress until the tooth is destroyed. It is defined as a localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates. It's an infectious disease with multifactorial etiology. The main responsible bacteria for this disease are the endogenous strains *Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus spp.*, present in the biofilm. The acidic metabolites cause a local pH fall below a critical value (pH 5.5) resulting in the demineralization of the tooth tissue.[1, 2] The demineralization process consists in dissolution of the hydroxyapatite crystals, the major component of tooth enamel and dentin, by organic acids that diffuse into the tooth. However, the tooth tissue can be remineralized by calcium, phosphate, carbonate, and fluoride present in saliva. Demineralization and remineralization happen simultaneously in the oral cavity for most of the people. The evolution of dental caries is dependent on a balance between both processes. Therefore, any factor that can push this balance toward the proceeding of remineralization can be utilized for the prevention of dental caries.[3]

Saliva is a clear, slightly acidic and complex biological fluid composed of secretions from major and minor salivary glands. Human salivary glands produce about 1-1,5 liters of serous and mucinous saliva daily by combining water, salts, and a variety of molecules from the blood with a cocktail of salivary proteins in the oral cavity to give rise to the multi-constituent whole saliva.[4] Saliva is considered an indicator of health and disease, because many of the compounds in blood are present in saliva. Therefore, saliva can be used to study the physiological state of the body.[5-7] It is considered a body fluid with a tremendous potential in the early detection and diagnosis of diseases.[5, 8, 9] This is possible largely due to the abundant protein content in saliva, which can behave as biomarkers for different diseases.[10]

Like blood, saliva is a complex biofluid filtered and processed from the vasculature of salivary glands. It contains a variety of enzymes, hormones, antibodies, antimicrobial constituents, growth factors and present multiples biomarkers of different diseases, like some cancers, autoimmune diseases, viral diseases, bacterial diseases, cardiovascular diseases and HIV.[9, 11] This fact allows the determination of mean levels of natural or depending substances, therapeutic molecules, hormonal status, immunological status, neurological effects, nutritional and metabolic influences. Furthermore, saliva is easy to collect, to store and to ship, and can be obtained at low cost in sufficient quantities for analysis. For patients, one of the most important

properties is that collection of saliva is a non-invasive method, which dramatically reduces anxiety and discomfort and simplifies the procedures of repeated sampling for longitudinal follow up.[5, 9]

The emerging biotechnology techniques contributed to the discovery of the salivary proteome that was a milestone for the study of potential biomarkers for various diseases.[5, 9, 10] The Human Salivary Proteome creates a kind of "periodic table" of the parotid, submandibular and sublingual secretory components, as well as it helps to elucidate some disease pathogenesis and to evaluate the influence of drugs on the structure, composition and secretion of all salivary secretory constituents.[8] Thus, besides protecting the oral cavity salivary proteins act as biological markers of general health and disease.[5] These latter may be derived from oral or systemic diseases having a significant role in the diagnosis properties of saliva.[12]

The salivary proteome is dynamic, because the synthesis starts by transcription and translation of the genes encoding the saliva proteins in the salivary glands, and is subsequently processed by various mechanisms such as glycosylation, phosphorylation, and proteolysis. When proteins enter the oral cavity, they may undergo further modification. In addition, new proteins are constantly entering the oral cavity via the salivary ducts, while others are formed by terminal processing in the oral environment and others are removed by swallowing. Thereby, the definition of salivary proteome is very variable, but it is important to recognize their composition for the salivary diagnosis.[13]

The post-translation modifications are important for the various functions of salivary proteins. For instance, the phosphorylated proteins have an important role in the state of supersaturation of saliva and in mineral homeostasis.[13]

In conclusion, the oral fluid, being the "mirror of body", appears as a very promising medium to be explored for health and disease surveillance. The translational applications and opportunities are enormous. Saliva can be used for detection of caries risk, periodontitis, oral cancer, breast cancer, salivary glands diseases and systemic diseases as hepatitis, HIV and HCV.[8, 10]

The present study aimed to compose a systematic review about the potential salivary biomarkers of dental caries.

3. Methods

Scientific manuscripts were searched in PUBMED, ScienceDirect, MEDLINE, BIOMED databases, which related salivary biomarkers with dental caries, using several keywords and MeSH terms. The review includes articles published in majority between 2000 and 2013, with content relevant for the present review.

Research in databases was performed with filters such as: type of publication, the availability of text, data publication, species and languages. The type of published including was: journal article, review, scientific integrity review and systematic reviews. The search was limited between 2000 and 2013. The priority was given to articles with free full text availability, and to studies performed in humans. The languages of the selected publications were English, French and Portuguese.

After the initial search there were found 120 papers, but after evaluating the title and abstract 75 of them were excluded. The reasons for excluding those studies were:

1. Not considered saliva as a potential medium for diagnostic use;
2. Studies performed in non-human animal models;
3. The absence of correlation of salivary proteins and the caries risk;
4. The results not comparing the protein level between the caries-free individuals and caries active subjects.

The remaining 44 articles were full evaluated and were divided into two groups. The group 1 includes the papers with general information about the saliva, such as, the potential of salivary diagnostic and the salivary proteome. This group included 22 articles, which were mainly used in the introduction, and in the topics about saliva. The group 2 included all the specific information of potential salivary biomarkers of dental caries. It contained 23 papers mainly used in the topics of potential biomarkers of dental caries and in each potential protein marker of dental caries.

4. Saliva

Saliva is the exocrine secretion of the major and minor salivary glands. It contains various proteins, enzymes, immunoglobulins, electrolytes, minerals, buffers, growth factors, cytokines, mucins, other glycoproteins and about 99% water. The components of saliva are important for its function of preserving and maintaining the health of oral cavity.[14-17]

Initially, saliva is an isotonic fluid, but in the final form it is hypotonic due to the absorption of sodium and chloride in ductal cells.[7, 18] When saliva crosses the ducts and enters in the mouth, it admixes with blood cells, gingival fluid, desquamated epithelial cells, food debris, microorganisms, nasal and bronchial secretions, forming the total saliva.[14-16, 18]

Saliva has various functions due a large diversity of proteins with a significant role in protection of oral tissues. Saliva plays a variety of roles such as lubrication, reparation of oral mucosa, formations and swallowing of food boluses, digestion, tasting, antimicrobial, buffering and reminalization.[7, 16, 17, 19-21]

The main salivary functions are:

1. **Taste** – saliva is isotonic when it is within the acini, but as it traverses the ducts it becomes hypotonic. It is the hypotonicity of saliva that allows the dissolution of substances in gustatory buds.[14]
2. **Protection and lubrication** – lubrication is exerted by mucins, proline-rich glycoproteins and statherins. The mucins of saliva form a seromucosal cover that protects, lubricates, prevent the dehydration and maintain the viscoelasticity of saliva. It also protects tissues against the proteolytic attacks of microorganisms.[14, 19-21]
3. **Dilution and cleaning** – the salivary flow dilutes the substances, cleans the oral cavity of carbohydrates, non-adherent bacteria, desquamated epithelial cells and food debris. This phenomenon is essential for decreasing the availability of sugars for the biofilm.[14]
4. **Buffer capacity** – the buffering capacity results by the action of the carbonic anhydrases, histatins, statherins and proline-rich anionic proteins. Buffer capacity of saliva by specific mechanisms as bicarbonate, phosphate and protein systems, reduces the acid of dental plaque and restore the pH for a level upper to critical value. The proteins of saliva act as a buffer system for the neutralization of acid products from bacteria metabolism. Therefore, the environment is unfavorable for the bacterial colonization. Saliva has two main buffer systems. The carbonic acid-bicarbonate system is the most efficient in

stimulated saliva. The main active system in unstimulated saliva is the phosphate buffer system.[14, 21]

5. **Maintenance of the integrity of teeth** – saliva modulates the phenomena of remineralization and demineralization. These phenomena are controlled by the concentration of salivary calcium, phosphate, fluoride, and pH. The presence of fluoride in saliva is very important because it reduces acid production in the biofilm.[14]

   Saliva promotes the remineralization of initial caries lesions, as a consequence of salivary supersaturation in free calcium, phosphate and fluoride ions.[21]

   Moreover, proline-rich proteins, statherins, histatins and cystatins bind to hydroxyapatite of enamel crystals and inhibit the precipitation of calcium and phosphate.[21]

6. **Digestion –** digestive function is accomplished by amylases, ribonucleases, proteases and lipases. Digestion is initiated in the oral cavity by the enzyme α-amylase, and their action is limited to the mouth. The α-amylase is referred to as a good indicator of the function of the salivary glands, and represents 40% to 50% of the protein content of saliva.[14, 20, 21]
7. **Tissue Repair** – due to the presence of epidermal growth factor in saliva.[14]
8. **Antibacterial properties** – the antibacterial function is conducted by peroxidases, lactoferrins, lysozymes, defensins, histatins, mucins, cystatins, immunoglobulins and proline-rich glycoproteins.[20]

   The main component of the immune saliva is secretory immunoglobulin A (IgA), which is able to neutralize viruses, bacteria, and enzyme toxins. It acts like an antibody that binds to bacterial antigens, promoting bacterial aggregation and inhibition of bacterial adherence to oral tissues.[14]

   Lysozyme is an enzyme that destroys the bacterial cell wall of some bacteria. Lactoferrin competes with various microorganisms in binding to free iron; this competition mechanism has a bacteriostatic and bactericidal effect on various microorganisms that depend on this ion to survive.[14]

   The statherins and proline-rich proteins play an important role in the inhibition of the spontaneous precipitation of calcium and phosphate ions in salivary glands and saliva, and in the formation of dental calculus.[14]

   The antimicrobial properties of saliva play an important role in the control of dental caries. This control is due to the maintenance of a proper equilibrium on oral ecosystem, due to the action of several proteins: proline-rich proteins, lysozymes, lactoferrins, peroxidases, agglutinins, histidines, sIgA, IgG and IgM.[21]

4.1. Salivary flow

Saliva contributes to the longevity of the teeth due to its protective and antimicrobial functions. [22] Since various decades, the dentists use the saliva for evaluation of the risk of dental caries through measurement of pH, flow and bacterial content.[20]

Saliva can influence the development of caries due to the salivary flow and content in fluoride. Thus, these factors play an important role in the prevention of dental caries.

The salivary flow is important in the prevention of caries, and it was established a high risk of caries in individuals with a low unstimulated salivary flow.[21] Cavities are most prevalent in patients with a lower salivary flow due to a decrease in the antibacterial, buffering and cleansing functions.[19] The salivary flow has influence in clearance of oral cavity by means of a reduction of bacterial and carbohydrates levels.

The hydration status of the mouth has influence in oral health, because the hydration is favorable to clearance, lubrication and swallowing. The salivary viscosity reduces the hydration capacity of saliva, and consequently raises the caries risk.

Many oral and systemic diseases have manifestations in salivary flow and composition of saliva. Treatments as radiotherapy affect the salivary glands, and these conditions result in alteration of quantity, quality and composition of saliva. Various medicines have an anticholinergic action, which reduces the salivary flow and may cause an alteration of salivary composition. The dentist has a duty to keep up to date on this topic.[14, 18]

4.2. Salivary proteome

The salivary proteome is composed mainly by proteins involved in oral biochemistry, and more than 1400 proteins were already identified. Due to its complex composition, this proteome can provide biomarkers of local and systematic diseases.[23]

The characterization of the human salivary proteome can help to understand the pathophysiology of diseases and monitoring the health or disease states. It may be possible to adapt the therapeutic effects based in the structure, composition and secretion of saliva from salivary glands.[19]

The characterization of the oral microbiota and salivary proteome associated with dental caries can be an important step towards the identification of biomarkers to caries and to predict caries susceptibility, allowing the intervention in a presymptomatic state. In fact, changes in salivary proteome can provide changes in microbial flora, and consequently lead to caries progression.[24]

4.3. Salivary diagnosis

The multi-components of saliva open the door to research of systemic diseases and monitoring general health.[20]

Saliva is used for diagnosis of systematic diseases because it is rapidly and easily collected, and is constituted by elements presents in serum that enter in oral cavity by the vasculature of salivary glands. These elements constitute the biomarkers which are used for diagnosis of systematic diseases. Saliva can be used for diagnosis of hereditary diseases, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, and for monitoring the therapeutic levels of medicines and illicit drugs.[18] Indeed, an analysis of saliva is a good mean for monitoring the level of substances as therapeutics, drugs and hormones, in plasma. Saliva appears as an alternative method of diagnosis, for the control of evolution of diseases and for monitoring the dosage of therapeutics.[21] Some potential salivary biomarkers were identified by a proteomic analysis for some diseases such as, for example, oral cancer, fibromyalgia syndrome and Sjögren's syndrome.[25]

Saliva may also be potentially used for the diagnose of HIV, breast cancer, diabetes, arthritis and heart disease, and it is expected to become a mean of diagnosis at the same level of blood or urine analysis.[19]

One important potential application of saliva as a diagnostic tool involves dental caries. Salivary diagnosis permits monitoring the efficacy of chemical treatment in the control of high risk caries patients, by the salivary quantification of the colonies of *Streptococcus mutans* and *Lactobacillus spp*. [21]

Moreover, subjects present a genetic variation in the protein composition of saliva, which can constitute a possible etiology factor of dental caries. The analysis of salivary protein content will be a form to predict dental caries risk in future, where those proteins will be used as biomarkers of disease.[26]

The identification of salivary biomarkers of dental caries is important for identification of individuals with risk to develop caries and institute a preventive treatment. The main difficulty is to determine just one variable to predict the risk of caries, because dental caries have a multifactorial etiology.[25]

Finally, the evolution of salivary diagnosis results in an important role of dentists in the diagnosis and monitorization of non-oral diseases.[18]

5. Potential biomarkers of dental caries

Saliva contributes to the longevity of the teeth due to its protective and antimicrobial functions. It is composed by various proteins essential for the oral health, because many proteins present antimicrobial activity. The concentration of a component of saliva may be variable over the day; this fact is influenced by glandular activity.[22]

The tooth surface is protected by a film formed by salivary proteins such as mucins and proline-rich glycoproteins. The proline-rich proteins and statherins help in the remineralization of enamel due to its ability to attract calcium.[22] In a study conducted in Colombia, it was observed that the salivary protein content was higher in women than in men. Furthermore, in females, more proteins were found in women with a history of caries than in women with active caries.[27]

5.1. Soluble Immunoglobulin A

Salivary antibodies are the first line of immune defense against antigens present in saliva, epithelial and tooth surfaces. Salivary immunoglobulins are produced by plasma cells present in stroma of salivary glands, adjacent to the salivary ducts and oral mucosa.[28, 29]

The main salivary immunoglobulin of whole saliva is the secretory immunoglobulin A (sIgA), which is constitutively secreted into saliva and is considered the first line of defense against microbial invasion, due to its ability to inhibit bacterial adhesion to the epithelial cells.[20, 28, 29]

More precisely, the immunoglobulins sIgA play an important role against the pathogenesis of dental caries, causing aggregation to specific bacterial proteins, consequently leading to agglutination and inactivation of bacterial enzymes and toxins. In addition, it promotes the inhibition of bacterial adherence, by the reduction of the hydrophobicity of bacteria. Also, it seems to act synergistically with other defense mechanisms, such as the lactoferrins, peroxidases, agglutinins and mucins.[28, 30-32]

The normal level of sIgA in individuals without systemic or immunological diseases ranges from 4-30 mg/dL. [32] This level is changed by numerous conditions, like malnutrition, obesity, infections, stress, smoking, salivary flow rate, hormonal factors, emotional states and physical activity. [29, 32] In elderly people, a decreased level of sIgA is associated with an increase in root caries and candidiasis.[20]

In an Indian study of Chawda et al [32], thirty children were evaluated, and three groups were formed according with their decayed, missing, or filled teeth (DMFT) for permanent teeth and/or decayed, or filled teeth (df-t) for deciduous teeth scores: low caries activity (DMFT and or df-t =1-5), high caries activity (DMFT and or df-t =6-10) and caries free (DMFT and or df-t =0). Each group was composed by ten children. The three groups have their levels of sIgA between 11-32 mg/dL, which fell within the normal range (4-30 mg/dL). In this study, it was observed that the total salivary concentration of sIgA was higher in caries free children than in the other groups with active caries. It was suggested the possibility of salivary sIgA antibodies may play an important direct role in the immune response against the pathogenesis of dental caries.[32]

In a study of Omar et al [31] realized in Egyptian Preschool Children, fourty children were selected and divided in four groups according with their decayed, missing and filled teeth (dmft) for deciduous teeth scores: low caries experience (dmft=1-3), moderate caries experience (dmft=4-6), higher caries experience (dmft>6), and caries free (dmft=0). The results of this study demonstrated that the sIgA levels decreased with the increase of the caries lesions. However, the group with the lower caries experience has a higher level of sIgA those caries free children, except the group with the lower levels of caries had a higher level of sIgA than the caries free group. This phenomenon can be explained by a protective immune response against an initial caries attack. In overview, the levels of sIgA appeared to be inversely proportional to dmft scores, which suggest a protective role of salivary immunoglobulin A against dental caries.[31]

In a study of Vitorino et al [33], thirty two male individuals were observed and divided in two groups according with their DMFT scores: caries free group (DMFT=0) and caries susceptible group (DMFT=3-12). Each group was composed by sixteen subjects. The results obtained demonstrated that the IgA was present at higher concentration in caries susceptible group. Furthermore, a positive correlation between levels of IgA and caries susceptible groups was observed.[33]

Taken together, these studies revealed different correlations between the salivary IgA levels and caries. The two first studies found a negative correlation, being observed lower levels of sIgA in the higher caries susceptibility groups. However, in the last study, it was observed a positive correlation, which associated the higher levels of IgA with higher caries susceptibility.

In this context, it is important to highlight that the caries susceptibility was measured by the DMFT index, this score give information of past and present dental caries. For the evaluation of dental health, at the time of evaluation, the DMFT index is incapable to distinguish the present

experience from the past experience of dental caries. The division of subjects in groups such as low caries experience, moderate caries experience and higher caries experience is not unanimous, and the variations observed in different studies may contribute to the contradictory results found in the literature.

5.2. Mucins 1 e 2

Mucins are glycoproteins produced by submandibular, sublingual, labial and palatinal minor salivary glands. These proteins are the main constituents of the mucus that covers the entire mucosal surface with a viscoelastic layer with 10-22μm thick at minimal. Such layer imprisons microorganisms and antigens, which are then eliminated by the washing action of salivary flow and swallowing. They have an important role in the concentration of other antimicrobial proteins in oral mucosa, such as the lysozyme, IgA and cystatin. The mucins are present in acquired pellicle from tooth surfaces and protect teeth surface from demineralization.[28, 29]

Saliva contains two forms of mucins, the high-molecular-weight mucin glycoprotein-1(MG1 or MUC5b) and the low-molecular-weight mucin glycoprotein-2 (MG2 or MUC7). The MUC5b has a molecular weight greater than 1000 kDa, while the MUC7 displays a molecular weight of 180-200 kDa.[28, 29]

The mucins are proteins with an important function in the protection of oral surfaces. They also control the processes of demineralization and remineralization, as demonstrated by the fact that when their levels are decreased, the prevalence of dental caries raise.[26]

The protective role of mucins against dental caries is reported in several studies. For example, in México, it was observed that the individuals with a higher DMFT index (>10.0) show a decrease or total absence of MG1, MG2 and acidic proline-rich protein-1, compared to subjects with lower DMFT index (≤4.0).[26] Patients with DMFT index of 11.87 presented lower number of MG1 and MG2, while subjects with DMFT index of 10.0 had less proline-rich protein-1.[26] A correlation between the quantity of proteins MG1 and MG2 and DMFT index was observed, where the absence of 6-13% of these mucins was associate to a higher DMFT index.[26]

5.3. Cystatin S and Statherin

Saliva presents seven different cystatins, cystatin A, cystatin B, cystatin C, cystatin D, cystatin S, cystatin SA and cystatin SN.[16, 28]

Cystatin S (AA1-8) variant, is a truncated form of cystatin S formed by proteases which cleave the first eight N-terminal amino acids, on the carboxyl side of arginine. These proteins are cysteine protease inhibitors, and are mainly present in submandibular saliva.[28, 34] The capacity of oral protection of Cystatins is thought to be related to the inhibition of cysteine proteases, namely cathepsin B, C, H and L.[33]

Cystatin S can be phosphorylated in five sites. The phosphorylated forms have an important function in the regulation of calcium levels and in the pellicle formation. The removal of the phosphate groups of cystatin reduces the affinity of the protein to hydroxyapatite.[34]

In a study of Vitorino et al [33], thirty two male individuals were observed and divided in two groups according with their DMFT scores: caries free group (DMFT=0) and caries susceptible group (DMFT=3-12). Each group was composed by sixteen subjects. The whole saliva of caries free group had a high concentration of Cystatin S, SN1, SN2 and SA-III, which was no longer present in the group susceptible to decay. After Spearman correlation coefficients analysis, it was observed that the DMFT had a negative correlation with several proteins from whole saliva, including cystatins, acidic proline rich proteins and lipocalin-1.[33]

Statherin is a protein with a molecular weight of 5.4 kDa, which has many functions, the most important being the inhibition of precipitation in supersaturated solutions of calcium. Therefore, it is the primary regulator of mineralization in the oral cavity. This characteristic is due to the negatively charged phosphorylated N-terminal.[28, 34]

The salivary levels of statherin and variant cystatin S (AA1-8) represent the best way to divide patients in high aggregation-adherence and low aggregation-adherence individuals. These proteins have an inverse correlation with occlusal caries. Higher levels of statherin and cystatin S are observed in caries-free individuals.[34]

Several studies have revealed that statherin competes with *Streptococcus mutans* in the binding to other proteins. This protein binds to other salivary proteins, forming heterotypic complexes exposing the bacteria to antimicrobial action of other salivary proteins.[34]

A study of Rudney et al [34] investigated the influence of bacterial aggregation, adherence and killing in the risk of dental caries. This research divided the subjects in four groups, namely, low aggregation-adherence/low killing, low aggregation-adherence/high killing, high aggregation-adherence/low killing and high aggregation-adherence/high killing. [34] The results

demonstrated a reduction of caries in groups with high aggregation-adherence. These groups had higher levels of cystatin S and statherin. However no differences were observed in groups with high or low killing of bacteria.[34]

Taken together, the levels of cystatin S and statherin can serve as potential indicators of risk for the development of dental caries.[34]

5.4. Defensins

Saliva has various antimicrobial peptides important for the innate immunity, namely the defensins. Defensins are small, cationic proteins with antimicrobial activity. The bacterial charge is an important factor for the susceptibility of bacteria to cationic peptides. These peptides are able to kill a variety of gram-positive and gram-negative bacteria, fungi and enveloped viruses.[35, 36]

Defensins can be divided in two subfamilies, including α-defensins and β-defensins. Both, the α-defensins (human neutrophil defensins - HNP) -1,-2,-3,-4 and the human β-defensins (HBD) -1,-2, -3, -4) are detected in saliva. It has been speculated that salivary α-defensins are produced by neutrophil and the salivary β-defensins derive from keratinocytes of oral mucosa.[28, 35] Defensins may be useful for the prevention of dental caries.[35]

The levels of HNP-1 are higher in saliva of patients with oral pathology, than in saliva of normal individuals. Although HNP-1 may be undetectable in salivary glands, the neutrophils migrate from blood through gingival crevicular fluid and mix with saliva, being speculated that salivary HNP-1 can be derived from neutrophils. These cells are activated when there is an inflammatory component in oral cavity. HNP-1 may be an important antimicrobial protein in innate immunity of mouth, appearing as the first line of defense against an infection.[35]

In a study of Ouhara et al [37], the β-defensins and cathelicidin (CAP18) LL37 revealed an antimicrobial activity against gram-negative and gram-positive bacteria, fungi and viruses, which appeared to be important for protection of the oral tissues.[37]

As stated above, it is believed that β-defensins are involved in innate immunity, acting as first antibacterial barrier. In line with this, the hBD1 is continuously expressed, whereas the other β-defensins are induced by bacterial contact.[37] The hBD1 and hBD2 act primarily on Gram-negative bacteria. The hBD3 is effective against gram-negative and gram-positive bacteria.[37] The antibacterial activity of hBD1 and hBD2 is less effective against Gram-positive bacteria. However, the HBD3 and LL37 have a greater antibacterial effect that the hBD1 and

2.[37] The protein CAP18 cathelicidin, when it is processed by proteases that activate the last amino acid (LL37), acquires antibacterial activity.[37]

The susceptibility of *S. mutans, S.mitis, S.salivarius, S. sanguis, S. sobrinus, L. casei* to antibacterial peptides, hBD1, hBD2, hBD3 and LL37 was evaluated previously. It was observed that all of the antibacterial peptides are bactericidal at concentrations above 10 mg / L, except the hBD1.[37] In the presence of saliva, the incubation of *S. mutans* with hBD1, hBD2, hBD3 and LL37 causes a reduction of their antibacterial activity, respectively 23%, 11% and no reduction for the last two proteins. The *S.mutans* was very susceptible to hBD3 and LL37.[37]

The study of Ozturk et al [36] analyzed the expression of three single-nucleotide polymorphisms of DEFB1and their relationship with the DMFT index. The DNA was extracted from saliva of 296 individuals. These subjects were divided in two groups, namely, low caries group and high caries group, in according with DMFT scores. The low caries group was formed by subjects with DMFT < 14 for subjects aged below thirty years, and DMFT < 9 for individuals with thirty or more years. The high caries group was composed by subjects with DMFT ≥14 for subjects aged below thirty years and DMFT ≥9 for individuals with thirty or more years.[36] The polymorphisms from DEFB1 studied were: rs11362 (G-20A), rs1800972 (C-44G) and rs1799946 (G-52A). They found that the rs11362 (G-20A) polymorphism was associated with a five-fold increase on the DMFT and DMFTS scores. In the rs1799946 (G-52A) polymorphism, it was observed a decrease of the DMFT index.[36] It was concluded that the GCA haplotype is associated with a low DMFT or DMFS, while the ACG haplotype is associated with a high DMFT or DMFS.[36] The polymorphisms of DEFB1 are a potential biomarker for caries risk.

5.5. CD14

CD14 is a protein with a molecular weight of 55 kDa, involved in innate immunity that actuates as a receptor of lipopolysaccharide (LPS) or peptidoglycan (PGN) of gram-negative and gram-positive bacteria, respectively. The LPS/PGN-CD14 complex binds to Toll-Like receptors of polymorphonuclear cells and activates the production of inflammatory cytokines by multiple signaling pathways. It mediates the activation of endothelial cells, epithelial cells and polymorphonuclear leucocytes. CD14 is expressed in cell surface of monocytes, macrophages and neutrophils, via a glycosylphosphatidylinositol anchor, and is present in plasma in the soluble form, sCD14. Major salivary glands secrete sCD14 into saliva. sCD14 acts as an important anti-cariogenic factor. It enables the binding between the epithelial cells and bacteria

and activates the production of cytokines for the recruitment of phagocytes. This protein mediates the activation of endothelial and epithelial cells, which are CD14-negative cells.[38-41]

In a study of Bergandi et al [38], it was conducted a immunoblotting analysis of saliva from 20 healthy children caries free and 20 children with active caries. In caries-free children, it was observed the presence of soluble CD14 (sCD14) using the anti-CD14 human immunoglobulin, while, in children with dental caries, this protein was absent. A second analysis was realized in 10 out of the 20 children with caries after 20 days to 6 month of restoration of carious lesions, and it was observed the presence of sCD14 in saliva. After the same period of time, a second check was realized also in 10 children without caries and their saliva continues to express sCD14. The absence of CD14 in saliva can be a potential biomarker of dental caries. The absence of sCD14 in saliva of caries active children seems to be a consequence of dental caries.[38]

In the Patent application, untitled "Use of the salivary protein CD14 as an indicator of the low risk to developing dental caries", it was considered the existence of ongoing caries when there was an absence or a reduction of at least 20% of a predetermined threshold of sCD14. CD14 is considered a biomarker for the development of dental caries. An inverse relationship is observed between the presence of sCD14 in saliva and caries lesions. The proteins sCD14 may play an important role in prevention of dental caries, if used as biomarkers. [42]

5.6. Glucosyltransferases

The dental caries is an infectious disease and studies indicate that can be preventable with mucosal immunization.[43] Glucosyltransferases (GTFs) from *S. mutans* are a candidate for the production of dental caries vaccine. These proteins are virulence enzymes important for the synthesis of glucans. These glucans serve as a binding site for *streptococci* and others microorganisms. Glucans participate in oral colonization and formation of the oral biofilm.[43-45]

The enzymes GTFs synthesize adhesive glucans from sucrose present in oral cavity, being these glucans crucial for the adhesion of *S.mutans* to tooth surface.[31, 44] The *S. mutans* have three types of GTFs with different functions and localization. GTF B and GTF C are associated to cell wall, while the GTF D is secreted.[43] GTF B has a molecular weight of 148 kDa and produces soluble glucans, which play an important role in the development of dental caries. This fact is due to the role of glucans in adhesion of *S. mutans* in tooth surfaces. The saliva level of GTF B is strongly correlated with caries in young children.[31, 45] GTF C has a

molecular weight of 138 kDa and synthesizes soluble and insoluble glucans.[31, 45] GTF D is an enzyme with a molecular weight of 143 kDa which forms soluble glucans.[31, 45]

GTF B and C are most important for the adhesion phenomena than GTF D. Some studies claim that the GTF C plays an essential role in adherence and colonization. The levels of salivary antibody anti-GTF C are higher in caries free group than caries active group.[43]

GTF D as secreted and has the capacity to neutralize the IgA or IgG that present in saliva.[43]

In a study of Omar et al [31] realized in Egyptian Preschool Children, fourty children were selected and divided in four groups according with their decayed, missing and filled teeth (dmft) for deciduous teeth scores: low caries experience (dmft=1-3), moderate caries experience (dmft=4-6), higher caries experience (dmft>6), and caries free (dmft=0). The GTF level was determined by ELISA assay. This study demonstrated that the children with a greater DMFT scores had a higher GTF B level and concluded that the GTF B levels increased with the increase of caries experience. GTF B is a potential biomarker for the prediction of caries experience in children, but further longitudinal studies have to be developed.[31]

In a study of Vacca-Smith et al [45], it was evaluated the saliva of fifty children. These children were divided in two groups, caries free and early childhood caries each group with twenty-five children. The levels of GTF enzymes were determined using an ELISA assay. The results demonstrated a great correlation between the presence of caries and a high level of GTF B.[45]

The GTF-inhibiting (GIF) factor seems to have an important role in the protection of oral tissues from invasion of *S. mutans*. This protein is expressed in submandibular and sublingual saliva, but is absent in parotid saliva. GIF is a high-molecular-weight glycoprotein-α-amylase complex able to inhibit GTF from *S.mutans*. Consequently, it helps in the control of *S.mutans* colonization. GIF inhibits the capacity of glucans production by GTFs. The binding of GIF to glucan binding region of GTF, resulting in an inhibition of enzymatic activity, consequently reduces the cariogenic capacity of *S.mutans*.[44]

The GIF appears to play an important role against the colonization by *S.mutans*, in young children, because participates in innate defense before the devolvement of adaptive immunity.[44]

6. Conclusion

The dental caries affect the salivary proteome. Consequently saliva appears to be a potential source of biomarkers for dental caries. After the analysis of several studies, there are various proteins candidate for a biomarker role of dental caries, namely, sIgA, mucins 1 and 2, cystatin S, statherins, defensins, CD14 and glucosyltransferase B.

The caries susceptibility is measured by the DMFT index, which gives information about the individual caries experience in the past and present. For the assessment of dental health at the time of the study, the DMFT fails, since it cannot distinguish the present experience from the past experience of dental caries. The criteria for dividing individuals into groups with low, moderate and high caries experience are not uniform, varying from study to study, which may contribute to conflicting results.

Further studies are needed to define a benchmark to infer whether the individual has an increased risk of caries for an actuation in an early stage. This would require longitudinal studies and use the International Caries Detection and Assessment System (ICDAS) for dividing individuals into well-defined groups, active caries subjects, subjects with incipient carious lesions and caries free individuals.

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